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IMPACT OF ANTIDOTE MEDICINAL PLANT- *CORALLOCARPUS EPIGEUS* EXTRACT ON LIPID PEROXIDATION INDUCED BY *NAJA NAJA*- SNAKE VENOM IN ALBINO RAT

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ABSTRACT

Snake bite is a serious health hazard occurs throughout the world especially in tropical countries like India. This problem is more severe in the rural parts. In recent times due to the increasing realizations of health hazards and toxicity caused by synthetic drugs there has been a renewal of interest in the use of plant based drugs throughout the world. Almost 80% of the people in the developing countries depend on traditional medicines for primary health care including snake bites, most of which are derived from plants. Antiserum is the only therapeutic agent available against the snake envenomation. However antiserum sometimes does not provide enough protection against venom induced haemorrhage, necrosis, nephrotoxicity and often develop hyper sensitivity reactions. In order to overcome these drawbacks a search for suitable antagonist especially from plant sources is ventured throughout the world. Study on the role of *Corallocarpus epigeaus* on *Naja naja* venom induced lipid peroxidation in rats was attempted to identify its antidote activity against snake venom.

KEYWORDS: Antidote, Antivenom, MDA (Malanaldehyde), LPO (Lipid Peroxidation), Medicinal Plants, Traditional Medicine, *Naja naja* Venom

INTRODUCTION

Snake bite is an important cause and is one of the major health risks in many countries (Venkatesan *et al.,* 2013*;* Williams *et al*., 2010). The global snake bites exceeding 5,000,000 per year (Mariane *et al*., 2011). The antivenom activity of medicinal plants could be considered as an effective alternative to produce mammalian antibody for the treatment of snakebite envenomation (Meenatchisundaram *et al.,* 2008). The usefulness and to evaluate the antivenom properties of the Indian medicinal plants in management of poisonous snakebites (Chidambaram Kumarapppan *et al*.,2011). The usage of natural medicine identified from plants and herbs has been influenced by inadequacy of biomedical health system and due to its cultural acceptability and cost effectiveness (Ramar Perumal Samy *et al.,* 2013; Gupta and Peshin 2012). In folk medicine, many vegetal species are employed for the treatment of snakebites in communities that lack prompt access to serum therapy especially in developing countries (Carvalho *et al.,* 2013). Active compounds of higher plants and herbs have great impact on treatment and management of poisonous snake bite Atul Kaushik *et al.,* (2013).

Providing safe, readily available and effective treatment is one of the ways to mange poisonous snakebites to avoid harmful effects caused by venom. Important remedy is to search for different venom inhibitors from natural plant and herbal compounds and it plays significant role in cure and prevention of poisonous bites. Snake venoms contain many substances such as toxins, activators and inhibitors enzymes, growth factors and is a complex mixture with a wide spectrum of biological activities (Mady, 2002; Badr *et al*., 2012; Omale James et al., 2013). They alter the cellular and

enzyme activities of different organs and lead to different metabolic disorders. Lipid peroxidation is an important effect induced by snake venom plant extracts shown neutralization effect to some extent.

The role of free radicals in the development of certain disorders for example in inflammation, cancer, tissue injury following cardiac arrest, transplant surgery or certain type of chemical poisoning is attracting considerable interest. A free radical is most easily considered simply as an atom or group of atoms containing an unpaired electron. In theory therefore, radicals might be readily formed by hemolytic cleavage of normal two electron covalent bonds for example, it as is the usual convension, (Robin wilson, 1987) the free radical is depicted with a bold superscript dot then

$H_2O = H^{\cdot}$ and $OH^{\cdot}$

$H_2O_2 = HO_2^{\cdot}$ and $H^{\cdot}$ or $H_2O_2 = 2OH^{\cdot}$

The O_2 derived free radicals are involved in the pathogenesis of rat gastric mucosal injury induced by haemorrhagic shock. In rats subjected to stress the reduction of gastric mucosal blood flow is regarded as an important factor in the formation of injury. In this context the injury to tissues by the stress has been reported due to involvement of free radicals (Itoh *et al*., 1987). In principle it appears as if stress borne out of drugs, environmental agents leading to tissue injury is of common and so does the case of the venoms whereby multitudinal toxicity of snake venoms in experimental models has been shown to involve tissue / cellular damage where the venom may induce the production of free radicals especially in the form of lipid peroxides (Ali *et al*., 1981). In view of the role of lipid peroxides in causing cellular / tissue injury attempt was made to measure the rat tissue Malanaldehyde (MDA) levels in LD_{50} dose and antidote plant aqueous and ethanolic extracts separately and in combination treated rats in order to check whether the extracts screened can potentiate snake venom altered rat tissue MDA levels *in vivo*.

MATERIALS AND METHODS

Plant Extracts

Plant material was procured from different places using the area of study. Collected samples were immediately chopped into small fragments to facilitate shade drying. Shade dried plant materials were powdered mechanically and extracted using solvents. Plant extracts both aqueous (AE) and ethanol extract (EE) with different concentrations (dose) were prepared from *Corallocarpus epigeaus* (*C. epigeaus*) plant material (Weniger, 1991) to study its antidote activity.

Snake Venom

Commercial snake venom *Naja naja* was obtained from Haff-kine Institute, Bombay, India.

Maintenance and Treatment of Experimental Animals

A colony of albino rats were maintained in well cleaned and sterilized cages in the animal house and ambient temperature was maintained at 24^0C to 27^0C to minimise the effects of stress. Animals were fed with commercial rat feed supplied by Kamadhenu agencies, Bangalore, India. Rats weighing 150 ± 10 grams were used for the experimental studies. Rats were fasted for 24 hours prior to experimentation and were given free access to water. In all *in vivo* studies rats were treated with LD_{50}dose of *Naja naja* venom which was dissolved in saline medium, saline and *Corallocarpus epigeaus* (*C.epigeaus*) plant extract, individually treated group of rats acted as control ones, 24 hours after treatment the control and experimental group of rats were anaesthetised with ether and were dissected, collected tissues like brain, heart, liver and kidney were isolated quickly and used for further studies.

Lipid Peroxidation

In control and experimental tissue crude homoginates, the lipid peroxides (LPO) was estimated as malondialdehyde (MDA) released with thiobarbeturic acid (TBA) reaction using the procedure as described by Ohkawa *et al*., (1979).10% homogenate were prepared in ice cold 1.5% KCl by using a Teflan potter - Elvenhjem homogeniser. Samples were centrifuged at 2500 x g for 10 min. and the resulting supernatant was used for analysis.

Statistical Analysis of the Data

The experimental data was analysed statistically following the methods adopted by Pillai and Sinha (1968).

RESULTS

The data shown in table: 1 depicts that the LD_{50} dose of *Naja naja* venom treated rat tissues like brain, heart, liver and kidney exerted elevated levels of their MDA levels and the changes were found to be statistically significant ($P< 0.05$) compared to their control ones. The experimental control group of rat tissues receiving 50-150 mg/kg of *C.epigaeus* aqueous and ethanolic extracts exerted no significant changes over the control group of rats (saline treated ones). But dose dependent changes of both aqueous and ethanolic extracts with regard to tissue MDA levels *in vivo* were observed for individual extracts tested in this study. The percent changes observed over envenomation were shown for*C.epigaeus* plant aqueous and ethanolic extracts. Of all the plant extracts screened in varied doses the *C.epigaeus* ethanolic extracts were found to normalise the LD_{50} - does Naja naja venom altered MDA levels in rat tissue and the least doses were observed for *C.epigaeus* ethanolic extract alone (table 1).

DISCUSSIONS

Lipid peroxidation involves the direct reaction of oxygen and lipid to form free radical intermediates and semi-stable peroxides. Lipid peroxidation is damaging process because of the subsequent reaction of free radicals that are produced (Pappel, 1970). Also, subcellular membrane damage has been related to lipid peroxidation (Bishayee and Balasubramaniam, 1971; Klassen and Plaa, 1969). EL - Asmar *et al*., (1979) have reported that the increase in polyunsaturated fatty acids following envenomation may lead to an increase in the rate of lipid peroxidation, which might be responsible for tissue damage.

The LD_{50} dose of *Naja naja* venom treated rat tissues showed elevated levels of MDA over a period of 24 hours and the changes were found to be statistically significant ($P<0.05$). Aqueous and ethanolic extract treated experimental control group of rats (*C. epigaeus* aqueous and ethanolic extract treated group of rats) showed a difference of only ±10% over their control (saline injected) group of rats. The *C.epigaeus* aqueous extract in doses of 50-150 mg tested *in vivo* did not alter much of venom elevated MDA levels of rat tissue (table: 1).

The *C.epigeaus* ethanolic extract in concentration of 150 mg could able to reduce venom altered MDA levels of rat brain upto 26.01%, heart MDA level upto 19.30%, liver MDA levels upto 19.08% and that of kidney MDA levels upto 25.91%.The *C.epigaeus* ethanolic extract (100 mg dose) in rat tissue MDA level was reduced to some extent and the percent changes ranged in between 10% - 20% (table: 1). The overall data obtained confir that the ethanolic extracts of the plants at doses tested only at higher doses able to neutralize the venom effects with special reference to rat tissue MDA levels and the trend was observed more for *C.epigaeus* ethanolic extract.

The amount of MDA produced, as measured by the thiobarbutyric acid assay, is a good indicator of endogenous lipid peroxidation (Tappel and Azalkin, 1960). Changes in lipid metabolism following snake and scorpion venom have been observed by several authors (Condrea and De Vries, 1965; EL - Asmar *et al*., 1972; Zwaal *et al*., 1973; Rosenberg, 1976).

Increase in lipid peroxidation in various organs following venom administration might be due to availability of fatty acids which are mobilised from adipose tissue (Yaron and Braun, 1970; EL-Asmar *et al*., 1979). Khan *et al*., (1980) observed depletion of adrenal ascorbic acid following the administration of snake venom in rats, suggesting that the animal suffered significant stress. Moreover, increase in catecholamines following venom administration is well documented (Ismail *et al*., 1972; Henriques *et al*., 1968) and this through the adenyl - cyclase-cyclic-AMP, may cause lipolysis in adipose tissue. Levine *et al*., (1964) suggested that a direct lipolytic action of the venom may be due to increase in cyclic AMP concentration. Moreover, increased fatty acid liberation by envenomation would result in an increase in the acetyl CoA, leading to an increase in the synthesis of cholesterol (Ashmore and Weber, 1968). Ali *et al*., (1981) studied the effect of Russell's viper (*Vipera russelli*) on rat tissue MDA levels and these authors have reported increased MDA levels in rat major tissues upon envenomation over a period of 24 hours. They further reported the above mentioned reasons as responsible for enhanced envenomated rat tissue MDA levels. Like reasons may be responsible for the present observed trend of rat tissue elevated MDA levels upon LD_{50} dose of *Naja naja* venom treated rat tissues and the lipid peroxide formed in excess in envenomated tissue may cause cellular / tissue damage.

From the data presented in table – 1, the ethanolic extracts of *C.epigaeus* in doses tested can neutralize the venom effect to some extent by depleting the extent of envenomated rat tissue MDA levels and thus may reduce the extract of envenomated rat tissue / cellular injury by lipid peroxides. Usually antioxidants are known well to reduce the formation of excess MDA and whether the plants ethanolic extracts possess antioxidatant properties or not is not known due to lack of adequate experimental data to the present.

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Table 1: Effect of *Corallocarpus epigaeus* (Rottl) Clarke. Aqueous (AE) and Ethanolic Extracts (EE) on *Naja naja* Venom Induced Lipid Peroxidation and MDA Levels in Rat Tissue (Values Expressed as Nm of MDA/Gm Wet Wt of Tissue)

Name of Tissue	Control (Saline Treated)	Envenomated (LD_{50} Dose Treated)	Control+ C.Epigaeus – AE (50mg)	Control+ C.Epigaeus - AE(50mg)+ Venom	Control +C.Epigaeus – AE(100 mg)	Control+ C.Epigaeus- AE (100 Mg)+Venom	Control +C.Epigaeus- AE(150 mg)	Control +C.Epigaeus - AE(150 Mg)+Venom	Control +C.Epigaeus- EE(50 mg)	Control +C.Epigaeus - EE(50 Mg)+Venom	Control +C.Epigaeus - EE(100 Mg)	Control+ C.Epigaeus - EE(100mg)+Venom	Control+ C.Epigaeus- EE(150Mg)	Contro +C.Epigaeus- EE(150 Mg)+Venom
Brain SDPC	192.34± 1.24	272.91*± 4.9141.88	191.75± 1.26 0.306NS	254.96** ±3.16 6.57	195.61± 0.92 -1.700 NS	241.67** ±2.36 11.44	195.22 ±2.64 -1.497 NS	232.02** ±1.14 14.98	196.36 ±2.34 -2.09 NS	250.10** ±1.05 8.35	193.14 ±1.24 -0.415 NS	225.55** ±2.36 17.35	194.22 ±1.64 -0.977 NS	201.90** ±3.11 26.01
Heart SDPC	250.41± 5.23	325.83*± 4.64 30.11	251.92±2. 54 - 0.603NS	301.81** ±4.68 7.37	251.42 ±4.08 -0.403 NS	295.72** ±3.49 9.24	253.69 ±3.61 -1.309 NS	291.63** ±3.72 10.49	251.19 ±1.67 -0.311 NS	300.26** ±2.51 7.84	250.00 ±0.94 -0.163 NS	291.01** ±3.40 10.68	252.05 ±2.95 -0.654 NS	262.82** ±3.17 19.30
Liver SDPC	357.19± 2.14	462.91*± 3.7429.59	352.73± 4.69 1.24 NS	422.43** ±05.60 8.744	358.35 ±5.71 0.324 NS	402.36** ±6.75 13.08	361.41 ±6.74 -1.181 NS	394.52** ±5.61 14.77	364.02 ±3.14 -1.912 NS	422.17** ±08.30 8.80	362.41 ±4.16 1.461 NS	406.03** ±03.45 12.28	361.51 ±2.32 -1.209 NS	370.41** ±02.46 19.98
KidneySDPC	209.34± 3.91	295.26*± 2.08 41.04	210.36± 4.12 - 0.487NS	242.81** ±4.92 17.76	212.1 ±2.64 -1.323 NS	238.36** ±5.73 19.27	207.52 ±3.19 0.869 NS	223.79** ±2.18 24.20	210.11 ±1.08 -0.367 NS	252.10** ±05.02 14.61	209.36 ±2.10 -9.55 NS	235.24** ±04.89 20.32	211.72 ±1.67 -1.136 NS	218.75** ±4.13 25.91

C.epigaeus: *Corallocarpus epigaeus*
AE: aqueous extracts and EE: ethanolic extracts
Each value is the mean ± SD of seven samples
PC: Per cent change over control / envenomated ones.
***P<0.05** (Over saline treated ones).
****P<0.05**(Over envenomated ones).
NS: Not significant.

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